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Nutritive value of corn silage in mixture with climbing beans

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ABSTRACT

Corn (*Zea mays* L.) for silage is a major forage source for dairy cows in the northern USA. It has high-energy density, but crude protein concentration is low. This study was conducted to determine the silage fiber characteristics and fermentation profile of monoculture corn or in mixture with one of three climbing beans. The experiment was conducted in two locations in 2004 and 2005. Three climbing beans, velvet bean [*Mucuna pruriens* (L.) D.C.], 'Rongai' lablab bean [*Lablab purpureus* (L.) Sweet], and 'Scarlet Emperor' scarlet runner bean (*Phaseolus coccineus* L.) were intercropped with corn at two corn densities (82.5 and 55 thousand plants/ha). All three beans were sown at the same density (82.5 thousand plants/ha). Corn in monoculture or in mixture with bean was harvested between 1/2 and 3/4 milk line. Two 1-L glass jar mini-silos per treatment per replicate plot were filled at a density of 500 g/L, eight jars per treatment, and stored for a minimum of 45 days at room temperature (∼22 °C). At the time of ensiling a 500-g fresh sub-sample was also taken for dry matter and initial characterization of the corn and corn–bean mixtures. Each silo was analyzed for fiber characteristics, pH, and fermentation products. Silage crude protein (CP) concentration on average increased 12.6% in the mixture with the addition of any of the beans, but neutral detergent fiber (aNDF) concentration also increased 8.8%. The pH was 0.06 units greater in corn–bean mixtures than monoculture corn, and lactic acid concentration was on average 6.2% greater in the corn–bean mixtures than monoculture corn. The addition of climbing bean to corn increased CP but also had an effect on fiber

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DM, dry matter; FAA, free amino acids; IVTDDM, *in vitro* true digestible dry matter; aNDF, amylase-treated neutral detergent fiber; NDFD, neutral detergent fiber digestibility.

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concentration and fermentation profile. However, the nutritive value of corn–bean silage was similar to corn silage, indicating that corn–bean silage mixtures could be used in dairy cow rations.

1. Introduction

Corn (*Zea mays* L.) for silage is a major forage source for dairy cows in the northern USA. It has high-energy value (NE_L = 1.45 Mcal/kg), but crude protein (CP) concentration is low (88 g/kg dry matter) (NRC, 2001). Because of that, corn silage is usually combined with other feeds, such as alfalfa silage, soybean meal, and fishmeal for example, in total mixed rations (TMRs) to increase crude protein concentration. Additives such as urea have been used to increase the CP of corn silage and reduce protein supplementation to cows. Although urea increases the CP of the silage, urea hydrolyzes to ammonia, which slows the decline in pH and extends silage fermentation (Kung et al., 2003). The extended fermentation decreases dry matter (DM) recovery (Kung et al., 2003) and could reduce nutritive value of the silage.

Agronomic research has shown that intercropping grain crops with legumes is a viable strategy to increase crude protein and overall nutritive value of silages. Contreras-Govea et al. (2006) reported that intercropping kura clover (*Trifolium ambiguum* M. Bieb.) with winter wheat (*Triticum aestivum* L.) for silage increased water soluble carbohydrates compared with kura clover and CP concentration compared with monoculture winter wheat. In another study, corn was mixed at ensiling with one of 15 different legumes at a 50:50 ratio (Titterton and Maasdorp, 1997). Crude protein increased with all legumes, but neutral detergent fiber concentration increased and digestibility decreased compared with corn alone. Similarly, growing corn with soybean [*Glycine max* (L.) Merr.] or cowpea [*Vigna unguiculata* (L.) Walp.] increased CP concentration and decreased dry matter and hemicellulose concentrations of the silage (Eichelberger et al., 1997), without affecting dry matter yield (Carruthers et al., 2000). Anil et al. (2000) ensiled corn intercropped with one of three different forages, kale (*Brassica oleracea*), runner bean, and sunflower (*Helianthus annuus* L.) that represented 7, 16, and 27% of total DM. Crude protein, NDF, and lactic acid concentration increased in the corn–forage mixtures compared with monoculture corn. Dawo et al. (2007) ensiled corn and bean (*Phaseolus vulgaris* L.) intercropped at different densities. They did not find differences in pH with the addition of the bean compared with monoculture corn; however, CP, DM, and lactic acid concentrations did increase. Armstrong et al. (2008) reported that intercropping climbing beans with corn increased CP in the mixture, but also increased neutral detergent fiber concentration and decreased digestibility compared to monoculture corn. Based on this information it is clear that intercropping and ensiling corn with legumes is a feasible option to increase CP concentration in silage. However, it is important to identify the bean that best complements corn in the field and during ensiling because not all legumes ferment equally (Mustafa and Seguin, 2003; Owens et al., 1999). Therefore, the objective of this study was to determine the effect of growing corn alone or in mixture with one of three climbing beans on nutritive value and silage fermentation characteristics.

2. Materials and methods

2.1. Crop production

The forage used in this study was produced in summer 2004 and 2005 at the University of Wisconsin Arlington (43°18′ latitude N, 89°21′ longitude W) and Lancaster (42°50′ latitude N, 90°47′ longitude W) Agricultural Research Stations. Crops at Arlington were grown on Plano silt loam (fine-silty, mixed, mesic Typic Argiudoll), and at Lancaster on Rozetta silt loam (fine-silty, mixed, superactive, mesic Typic Hapludalfs). Soil fertility levels at both locations were maintained at optimal levels for corn silage production (Kelling et al., 1998). Corn was sown alone or in mixture with one of three climbing beans, velvet bean (speckled germoplasm from Sharad Phatak, University of Georgia), 'Rongai' lablab bean, and 'Scarlet Emperor' scarlet runner bean. Corn was sown in early May at two densities, 82,500 and 55,000 plants/ha, and beans were sown 2 weeks after corn in rows 15 cm from the corn rows at

82,500 plants/ha for all beans. Corn and corn–bean mixtures were harvested on 17 and 20 September in 2004 and 7 and 15 September in 2005 at Lancaster and Arlington, respectively, at a corn maturity stage of 1/2 to 3/4 kernel milk line.

2.2. Treatments for silage

Treatments included two corn densities (82,500 and 55,000 plants/ha) and three beans plus control (no bean) for a total of eight treatments with four replicates per treatment at each location both years. Monoculture corn and corn–bean mixtures were chopped with a conventional harvester to a theoretical particle size of 0.5 cm. Two 1-L glass jars per treatment per replicate were filled at a density of 500 g/L, eight jars per treatment, and stored for a minimum of 45 days at room temperature (∼22 °C). At the time of ensiling 500-g fresh sub-sample was taken for dry matter and initial characterization of corn and corn–bean mixtures. Silos were frozen to −20 °C to stop fermentation and remained frozen until silages were analyzed.

2.3. Fresh forage and silage analysis

Fresh and ensiled forage were analyzed for pH by placing a 20-g sample in a blender jar, diluting with deionized distilled water to 200 g, and blending for 30 s in a high-speed blender (Waring Commercial, Torrington, CT, USA. Model 7011S). The diluted sample was filtered through four layers of cheesecloth, and pH was measured immediately with a pH meter. A 20 mL aliquot was centrifuged at 25,106 × *g* for 20 min at 4 °C, and the supernatant was decanted into 20-mL scintillation vials and stored at −20 °C for later analysis of fermentation products. Organic acids (lactate, acetate, propionate, and butyrate) and ethanol were determined using high-performance liquid chromatography (Muck and Dickerson, 1988). The HPLC system consisted of a Shimadzu system controller (SCL-10A), pump (LC-10AT), and refractive index detector (RID-6A) (Shimadzu Corp., Kyoto, Japan) with a Bio-Rad Aminex HPX-87H column (Bio-Rad Lab., Hercules, CA) heated to 42 °C.

At the time of opening, duplicate samples (50 g) were taken for moisture determination by freeze-drying. After moisture determination, the duplicate freeze-dried samples were ground to 1 mm particle size and analyzed for CP by a Leco FP-2000A nitrogen analyzer (Leco Corp., St. Joseph, MI), sequential heat stable amylase and sodium sulfite-treated neutral detergent fiber (aNDF)–acid detergent fiber (ADF) batch procedures (Van Soest et al., 1991) inclusive of ash as outlined by ANKOM Technology Corp. (Fairport, NY), and *in vitro* true digestible dry matter (IVTDDM) determined using the Daisy II system (ANKOM Technology Corp., Fairport, NY). The aNDF digestibility (NDFD) in g/kg NDF was calculated from the aNDF and IVTDDM values (g/kg DM) where NDFD = 1000 × [aNDF − (1000 − IVTDDM)]/aNDF. Ammonia and free amino acids (FAA) were determined (Broderick et al., 2004) using flow-injection (Lachat Quik-Chem 8000 FIA; Lachat Instruments, Milwaukee, WI).

2.4. Statistical analysis

Data analyses were conducted using the Proc Mixed/REML Procedure of SAS (SAS Institute, 2001) as a factorial experiment 2 × 4 with four replicates by treatment per environment. Corn density, beans, and density × bean interaction were considered fixed effects. Environment (locations × year) and replications were considered random effects. When treatment effect was significant, means were separated using LSMEANS comparison (SAS Institute, 2001) at $\alpha = 0.05$ with the PDIFF option.

3. Results

Normal corn density had a bean proportion of 54 g/kg DM and low corn density had a bean proportion of 81 g/kg DM. Individual bean proportion was 54, 103, and 114 g/kg DM for scarlet runner, velvet, and lablab bean, respectively (Armstrong et al., 2008). Although the proportion of the bean in the mixtures was relatively low and adding beans did not have an effect on dry matter yield (Armstrong et al., 2008), there was an effect on nutritive value and silage fermentation characteristics compared with monoculture corn.

Table 1
Chemical composition of fresh corn and mixture forage grown at normal and low corn plant density

	Density effect		S.E.M.	P
	Normal	Low		
Dry matter (g/kg)	403	376	8.5	0.001
Crude protein (g/kg DM)	71	79	1.7	0.001
Neutral detergent fiber (g/kg DM)	320	329	7.6	0.262
Acid detergent fiber (g/kg DM)	161	170	3.5	0.082
Hemicellulose[a] (g/kg DM)	159	159	7.4	0.885
In vitro true digestible dry matter (g/kg DM)	813	803	5.1	0.163
Neural detergent fiber digestibility[b] (g/kg NDF)	420	404	24.3	0.094
Free amino acids (g/kg total N)	58	46	35.0	0.009
Ammonia (g/kg total N)	8	8	0.7	0.991

Values are pooled over four environments.
[a] Estimated from the difference of aNDF – ADF.
[b] Calculated from NDFD = 1000 × [aNDF – (1000 – IVTDDM)]/aNDF.

3.1. Corn–bean characteristics before ensiling

Corn density and corn–bean mixture characteristics before ensiling are shown in Tables 1 and 2. Density × bean interaction was not significant for any of the characteristics ($P>0.05$). Density effect was significant for DM, CP, and FAA concentrations. Low corn density had 27 g/kg and 12 g/kg total N lower DM and FAA than normal corn density. Among beans there were significant ($P<0.05$) effects for all characteristics except hemicellulose concentration. Lablab bean mixture had lower DM concentration (374 g/kg) than corn and corn–velvet bean and corn–scarlet runner bean mixtures. Monoculture corn and scarlet runner bean–corn mixture had similar characteristics, but the corn–velvet bean and corn–lablab bean mixtures had greater CP, aNDF, and ADF and lower IVTDDM and NDFD than monoculture corn. Free amino acids and ammonia-N were lower in corn–velvet bean than monoculture corn.

3.2. Corn–bean characteristics after fermentation

Trends in silage characteristics among treatments (Tables 3 and 4) were similar to fresh forages. The greatest effects of beans were observed on DM, CP, aNDF, and IVTDDM of mixtures (Table 4). Dry matter concentration was different between corn densities (Table 3) ($P<0.001$) and among beans (Table 4) ($P<0.001$). Low corn density had 6.4% lower DM concentration than normal corn density (Table 3), which appeared related to the greater proportion of bean in the low-density mixtures. Among beans,

Table 2
Chemical composition for fresh forage of corn in monoculture or in mixture with one of three climbing beans

	Species effect				S.E.M.	P
	No bean	Velvet bean	Lablab bean	Scarlet runner bean		
Dry matter (g/kg)	394	395	374	395	9.3	0.016
Crude protein (g/kg DM)	69	82	78	71	2.0	0.001
Neutral detergent fiber (g/kg DM)	316	340	339	304	9.6	0.004
Acid detergent fiber (g/kg DM)	155	176	180	151	5.0	0.001
Hemicellulose[a] (g/kg DM)	161	163	159	153	7.8	0.156
In vitro true digestible dry matter (g/kg DM)	819	794	796	823	7.1	0.003
Neural detergent fiber digestibility[b] (g/kg aNDF)	430	395	400	423	25.3	0.026
Free amino acids (g/kg total N)	52	40	53	62	4.7	0.011
Ammonia N (g/kg total N)	9	6	8	9	0.9	0.021

Values are pooled over four environments.
[a] Estimated from the difference of aNDF – ADF.
[b] Calculated from NDFD = 1000 × [aNDF – (1000 – IVTDDM)]/aNDF.

Table 3
Chemical composition of ensiled corn and mixtures grown at normal and low corn density

	Density effect		S.E.M.	P
	Normal	Low		
Dry matter (g/kg)	391	366	5.4	0.001
Crude protein (g/kg DM)	71	80	2.8	0.001
Neutral detergent fiber (g/kg DM)	368	372	12.1	0.486
Acid detergent fiber (g/kg DM)	190	199	4.4	0.027
Hemicellulose[a] (g/kg DM)	178	174	8.7	0.182
In vitro true digestible dry matter (g/kg DM)	788	778	6.7	0.095
Neural detergent fiber digestibility[b] (g/kg NDF)	423	404	24.0	0.054
Free amino acids (g/kg total N)	464	475	10.8	0.436
Ammonia (g/kg total N)	36	35	2.5	0.612

Values are pooled over four environments.
[a] Estimated from the difference of aNDF – ADF.
[b] Calculated from NDFD = 1000 × [aNDF – (1000 – IVTDDM)]/aNDF.

lablab bean had the greatest impact, decreasing DM concentration 6.9% compared to monoculture corn (Table 4). Averaging over corn–bean mixtures, DM concentration was 3.5% lower in the mixtures than corn alone. Even though there were DM differences, all treatments had DM concentrations within the range for good fermentation (Muck et al., 2003).

The amount of bean in the mixture also affected CP concentration. Crude protein concentration was 12.7% greater with low corn density than normal density (Table 3) ($P<0.001$). Among beans, velvet bean had the largest impact on CP (Table 4). The CP of that mixture was 13 g/kg DM greater than that of monoculture corn whereas scarlet runner bean and lablab bean increased CP by 4–9 g/kg DM, respectively. Only the increase in the scarlet runner bean mixture over corn alone was not statistically significant ($P>0.05$). Averaging over beans, the corn–bean mixtures had 12.6% greater CP concentration than corn alone.

The aNDF and hemicellulose (the difference between aNDF and ADF concentrations; Hatfield, 1993) concentrations were similar between corn densities ($P>0.10$), but ADF concentration was 4.7% greater in the low-density treatments (Table 3) ($P<0.03$). Averaging over beans, aNDF and ADF concentrations increased by 8.8 and 12.0% in the mixtures compared with monoculture corn. Among beans, the velvet and lablab bean mixtures had the greatest increases in aNDF and ADF concentrations compared with corn, which coincides with the fact that those two beans were a higher proportion of their mixtures than scarlet runner.

In vitro true digestible dry matter and aNDF digestibility were not different between corn densities (Table 3) ($P>0.05$), but IVTDDM was different among beans (Table 4) ($P<0.05$). Even though the IVTDDM

Table 4
Chemical composition of ensiled forage of corn in monoculture or in mixture with one of three climbing beans

	Species effect				S.E.M.	P
	No bean	Velvet bean	Lablab bean	Scarlet runner bean		
Dry matter (g/kg)	389	381	362	383	6.2	0.001
Crude protein (g/kg DM)	69	82	78	73	2.9	0.001
Neutral detergent fiber (g/kg DM)	347	389	395	349	12.9	0.001
Acid detergent fiber (g/kg DM)	177	208	212	180	5.2	0.001
Hemicellulose[a] (g/kg DM)	170	182	183	169	9.0	0.001
In vitro true digestible dry matter (g/kg DM)	798	768	770	796	7.8	0.001
Neural detergent fiber digestibility[b] (g/kg NDF)	417	403	415	419	24.9	0.630
Free amino acids (g/kg total N)	486	453	493	447	14.5	0.047
Ammonia (g/kg total N)	35	38	36	34	2.5	0.004

Values are pooled over four environments.
[a] Estimated from the difference of aNDF – ADF.
[b] Calculated from NDFD = 1000 × [aNDF – (1000 – IVTDDM)]/aNDF.

Table 5
Fermentation profile of corn and mixture silage grown at normal or low corn plant density

	Density effect		S.E.M.	P
	Normal	Low		
pH	3.97	3.99	0.04	0.292
Lactic acid (g/kg DM)	51.6	54.3	3.63	0.010
Acetic acid (g/kg DM)	10.9	11.9	0.67	0.006
Ethanol (g/kg DM)	5.4	4.9	0.55	0.194
Lactic acid:acetic acid ratio	4.8	4.7	0.09	0.207

Values are pooled over four environments.

was not different between densities, there was a trend ($P<0.10$) toward lower IVTDDM with low-density corn. Although there were no significant differences among beans and monoculture corn for NDFD, there was a trend for lower NDFD in the velvet bean and lablab bean mixtures than monoculture corn. Similar to IVTDDM, lower NDFD was observed on those mixtures with greater aNDF and ADF concentrations.

Ammonia and FAA concentrations were not different between corn densities (Table 3) ($P>0.05$,), but they were among bean mixtures (Table 4) ($P<0.05$). The corn–scarlet runner bean mixture had the lowest FAA (447 g/kg total N) and ammonia (34 g/kg total N) concentrations, while corn–lablab bean had the greatest FAA concentration (493 g/kg total N) and corn–velvet bean mixture had the highest ammonia concentration (38 g/kg total N). Averaging over beans, corn–bean mixtures had 4.5% lower FAA concentration than corn, but similar ammonia formation.

3.3. Fermentation products

Fermentation products (lactate, acetate, and ethanol) and pH are presented in Tables 5 and 6. Silage pH was not different between corn densities (Table 5) ($P>0.05$), but it was among corn–bean mixtures (Table 6) ($P<0.001$). Corn–velvet bean and corn–lablab bean silages had greater pH (4.02 and 4.01) than corn and corn–scarlet runner bean silages (3.93 and 3.95).

Lactic acid concentration was different between densities and among bean mixtures ($P<0.05$). Mixtures with low-density corn had 5.2% greater lactic acid concentration than normal density. Among beans, the corn–lablab mixture was 10.3% greater lactic acid concentration than monoculture corn, and 4.9 and 6.9% greater than scarlet runner bean and velvet bean, respectively. Averaging over bean species, the lactic acid concentration of the mixtures was 6.2% higher than that in monoculture corn.

Similarly to lactic acid concentration, acetic acid concentration was different between densities and among beans ($P<0.05$). Low corn density had 9.2% greater acetic acid concentration than normal corn density. Similar to lactic acid concentration, beans in the low-density corn had more extensive fermentation than normal density, perhaps due to the lower DM concentration of the low-density mixtures, allowing an increase in organic acid production. Among beans, the corn–lablab and corn–velvet bean silages had 18.9 and 12.4% greater acetic acid concentration than corn silage and corn–scarlet runner bean silages, respectively. Averaging over beans, the corn–bean mixture silages had 14.6% greater

Table 6
Fermentation profile of corn in monoculture or in mixture with one of three climbing beans

	Species effect				S.E.M.	P
	No bean	Velvet bean	Lablab bean	Scarlet runner bean		
pH	3.93	4.02	4.01	3.95	0.04	0.001
Lactic acid (g/kg DM)	50.6	52.2	55.8	53.2	3.70	0.005
Acetic acid (g/kg DM)	10.3	12.0	12.5	10.9	0.72	0.001
Ethanol (g/kg DM)	5.2	4.8	4.6	5.9	0.62	0.090
Lactic acid:acetic acid ratio	4.9	4.5	4.5	4.9	0.11	0.002

Values are pooled over four environments.

acetic acid concentration than corn silage. Ethanol concentration was not different either between densities or among beans (P>0.05).

4. Discussion

The DM concentrations of the corn–climbing bean mixtures relative to that of corn alone decreased as the percentage of legume increased. This appeared to be true whether the increase in legume was due to the ability of a climbing bean species to compete with corn or due to low corn density permitting greater bean growth. Similar reductions in DM concentration were reported in other studies mixing corn with kale (Anil et al., 2000) and soybean (Titterton and Maasdorp, 1997).

Combining corn with climbing beans increased silage CP, but also fiber concentration in silage. It was not a surprise that CP increased; previous research has shown that CP concentration increases when the proportion of corn decreases or bean increases in the mixture. Dawo et al. (2007) reported that CP concentration increased 22% in the mixture when corn proportion in the mixture decreased by 50%. Our results are in agreement with other studies where legumes also increased CP concentration when in mixture with corn (Anil et al., 2000; Dawo et al., 2007; Titterton and Maasdorp, 1997).

It is well known that cell wall components such as cellulose and lignin are greater in stems than leaves, and greater in legumes than grasses (Aman, 1993). Therefore, a greater proportion of beans in low-density compared with normal density corn could explain the higher ADF concentrations in the low-density mixtures. The higher fiber concentrations of the mixtures led to small reductions (up to 30 g/kg DM) in IVTDDM. Cell wall structural constituents, such as cellulose and lignin, are negatively correlated with digestibility (Hatfield, 1993), which agrees with our findings.

Significant differences in proteolysis during ensiling as indicated by free amino acid and ammonia formation were observed across the four bean treatments (corn alone plus the three mixtures). However, variation between these treatments appeared to be related to species characteristics rather than the amount of legume in the mixture. The lowest free amino acids and ammonia concentrations were in the scarlet runner bean mixtures whereas the highest free amino acids were in the lablab mixtures and highest ammonia in the velvet bean mixtures. The monoculture corn silages had intermediate concentrations of both. Variations in proteolysis among legumes were previously observed in other studies (Albrecht and Muck, 1991; Mustafa and Seguin, 2003) and agree with these results.

The effects of the bean mixtures on silage fermentation were in the directions expected. Legumes have greater organic acid concentrations than grasses; therefore, in general legume silages tend to have higher pH because of the higher buffering capacity caused by the organic acids (Albrecht and Beauchemin, 2003; Muck et al., 2003). Silage made from the corn–bean mixtures in our study had higher pH and contained greater lactic and acetic acid concentrations than that of monoculture corn. Anil et al. (2000) also reported an increase in lactic acid concentration when corn was ensiled in mixture with other legumes. According to them, a more intense fermentation resulted from the lower DM content of the mixture compared with monoculture corn.

Of the three beans in our study, lablab bean had the greatest effect on fermentation compared to monoculture corn, but final pH and end product profile were well within the range considered to be normal. The difference in pH between the lowest and highest treatment was only 0.09 (Table 6), and the highest pH values (4.02) in the corn–velvet bean mixture. Velvet and lablab bean mixture silages should be anaerobically stable and not susceptible to clostridial fermentation (Muck et al., 2003). Similarly the greatest differences in lactic acid, acetic acid and ethanol concentrations between treatments were 5.2, 2.2 and 1.3 g/kg DM (Table 6). Such small differences in fermentation products are unlikely to significantly affect animal performance; animal performance would more likely be affected by IVTDDM, fiber and CP differences among the treatments (Weiss et al., 2003).

5. Conclusions

Combining corn with climbing beans increased crude protein, but also fiber concentration in silage. The higher fiber concentrations of the mixtures led to small reductions (up to 30 g/kg DM) in *in vitro* true dry matter digestibility. Silage made from the corn–bean mixtures had higher pH and contained greater lactic and acetic acid concentrations than that of monoculture corn, but these differences were not likely of practical significance relative to silage preservation or animal performance. Of the three beans, lablab bean had the greatest effect on fermentation, but final pH and end product profile were well within the range considered to be normal. Therefore, the results generated from this study support the conclusion that climbing beans intercropped with corn add extra protein to the silage with inconsequential effects on fermentation characteristics.

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